Influenza A virus lures autophagic protein LC3 to budding sites

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Abstract: Autophagy, a cellular degradation pathway, can restrict or assist viral replication. In this issue of Cell Host Microbe, Beale et al. (2014) report that the influenza virus matrix protein 2 binds to the essential autophagy protein LC3 to presumably transport LC3-conjugated membranes to the cell surface for budding of stable viruses.

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Influenza A virus hijacks parts of the autophagy machinery to the cell membrane

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Summary

Autophagy, a cellular degradation pathway, restricts or assists viral replication. Influenza A virus was now found to utilize the molecular machinery of autophagy by matrix protein 2 binding to the essential autophagy protein LC3 in order to presumably transport LC3-conjugated membranes to the cell surface for budding of stable viruses.

Autophagy describes a group of cellular pathways that import cytoplasmic constituents into lysosomes for degradation (Mizushima et al., 2011). Of these, macroautophagy forms double-membrane surrounded vesicles, so-called autophagosomes, that engulf parts of the cytoplasm, even whole organelles, and transport them to lysosomes. In the formation of these autophagosomes more than 30 essential macroautophagy gene products of autophagy related genes (atgs) are involved. One of these, an ubiquitin-like molecule called Atg8 gets directly coupled to the forming autophagosome membrane, and is probably involved in its elongation as well as the recruitment of substrates into autophagosomes. Light chain 3 (LC3) proteins are a group of three mammalian Atg8 orthologues. LC3 mediates substrate recruitment to autophagosomes via binding partners that carry LC3-interacting regions (LIR). Among these are some proteins that are incorporated in organelles and deliver these for macroautophagy. Others, like p62/sequestosome 1, carry LIR motifs and ubiquitin binding domains or bind to glycosylation detecting proteins, like NDP52 (nuclear dot protein 52) binding to galectin-8, in order to anchor ubiquitinated substrates or sugar moieties in the cytosol to forming autophagosomes (Randow and Munz, 2012). Thus, LC3 attachment usually leads to incorporation into autophagosomes, followed by delivery to and degradation in lysosomes.

These mechanisms are also used during xenophagy, macroautophagic degradation of intracellular pathogens, including viruses. In particular, neurotropic infections with the RNA virus Sendai virus (SeV) has been shown to be restricted by macroautophagy (Orvedahl et al., 2010). The SeV capsid gets ubiquitinated in this process and binds to p62/sequestosome 1 for incorporation into autophagosomes. The ubiquitin-ligase SMURF1 (SMAD ubiquitination regulartory factor 1) supports macroautophagy of SeV and co-localizes with the viral capsid (Orvedahl et al., 2011). Similarly, another RNA virus, chikungunya virus (CHIKV), gets degraded by macroautophagy after binding to p62/sequestosome 1 (Judith et al., 2013). This is the predominant interaction of CHIKV with the autophagic machinery in mouse cells. However, in human cells, CHIKV RNA replication occurs at a protein complex which is bound to LC3 at the

trans Golgi network via NDP52 (Judith et al., 2013). The latter interaction favors CHIKV replication, which becomes the dominant function of the autophagic machinery in human cells. This example documents that successful pathogens might not only evade innate immune restriction by macroautophagy, but can also use its molecular machinery for their own benefit.

In this issue of *Cell Host & Microbe* another example for such a pathogen is investigated. The segmented RNA virus influenza A virus has been previously described to block macroautophagy, by inhibiting fusion of LC3 coupled vesicles with lysosomes (Gannage et al., 2009). This leads to a perinuclear accumulation of presumably autophagosomes. The viral protein that achieves this block in autophagosome maturation is matrix protein 2 (M2), the proton channel that is essential for both viral entry and budding from the cell membrane. Beale, Randow and colleagues now identified a LIR motif in M2 (aa91-94) that directs LC3 to the cell membrane, but does not influence perinuclear LC3 accumulation (Beale et al., 2014) (Figure 1). This represents the first pathogen encoded LIR motif and suggests that the virus has developed it for his own benefit. In order to exclude that this would lead to M2 or even virus particle degradation, M2 seems to have acquired an additional domain that prevents autophagosome fusion with lysosomes. The M2 mediated redirection of LC3 to the plasma membrane might serve the purpose of delivering LC3 conjugated membranes to the cell surface. Indeed, membrane consuming filamentous influenza A virus budding is not observed in the absence of the LIR motif in M2. Nevertheless, the viral titers that are produced in the absence or presence of LC3 redirection to the cell membrane are similar. However, when the authors tested the stability of the produced influenza A virus particles after prolonged time periods (1-2days) at room temperature, infectivity of particles that were produced from influenza A viruses with LIR deficient M2 was decreased. Although LC3 is not incorporated into budding influenza A viruses, the presented evidence suggests that the viral envelope is changed by the absence of LC3 coupled membranes at the cell surface and renders the resulting viruses less stable. How LC3

or LC3 coupled membranes alter influenza A virus envelope composition requires further investigations.

Several other viruses seem to use the membrane fusion machinery of macroautophagy for their replication and virus particle release. Among these are important human pathogens like polio-, hepatitis C- (HCV) and human immunodeficiency virus (HIV) (Dreux et al., 2009; Jackson et al., 2005; Kyei et al., 2009). Poliovirus was indeed the first virus for which perinuclear accumulation of autophagic membranes was decribed. Indeed, the poliovirus proteins 2BC and 3A are sufficient to accumulate LC3 coupled membranes (Jackson et al., 2005). These membranes seem to serve as scaffolds for the viral replication machinery and transport poliovirus particles to the cell surface for release. HCV and HIV also subvert macroautophagy for their replication and release, respectively. At least four essential autophagy components, including the LC3 membrane conjugation machinery were found to be essential for early translation of HCV proteins (Dreux et al., 2009). Once membrane rearrangement has happened and HCV infection is established the molecular machinery of macroautophagy is no longer required for the replication of this RNA virus. Apart from using autophagic membranes as replication scaffolds, some viruses also highjack them to get out of cells. For example HIV blocks autophagosome maturation with its Nef protein (Kyei et al., 2009). In infected macrophages this assists viral replication, presumably by supporting multivesicular body formation which could be one of the viral budding sites in this cell type. Thus, successful pathogens not only block their degradation by macroautophagy, but also utilize the respective molecular machinery to shape cellular membranes for their replication and budding needs.

These additional functions of the macroautophagic machinery, in addition to viral particle degradation, complicate strategies to therapeutically manipulate autophagy during viral infections. They would suggest that each virus' regulation of this pathway needs to be analyzed in order to predict if macroautophagy inhibition or stimulation should be therapeutically explored. In addition, the effect of macroautophagy on extrinsic immune control of viral infections needs to

be taken into consideration. For example, macroautophagy inhibition might restrict influenza A virus infection intrinsically in infected host cells, but both antigen processing for MHC presentation by antigen presenting cells and T cell expansion might be compromised by this treatment. Therefore, the type and delivery of autophagy modulators, possibly inhibitors to lung pneumocytes during influenza infection via inhalation, have to be considered for therapeutic intervention. With studies like the one in this issue of *Cell Host & Microbe* we are just beginning to grasp the layers of viral regulation of macroautophagy, which can be harnessed for treatment of viral infections.

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Figure legend

Figure 1: Influenza A virus redirects LC3B to the cell surface. Influenza A virus arrests autophagosome fusion with lysosomes via its matrix protein 2 (M2). This leads to perinuclear LC3B-coupled membrane accumulation. Via direct binding to LC3B M2 redirects LC3B and presumably the associated membranes to the cell membrane for filamentous influenza A virus budding, which results in more stable viral particles.

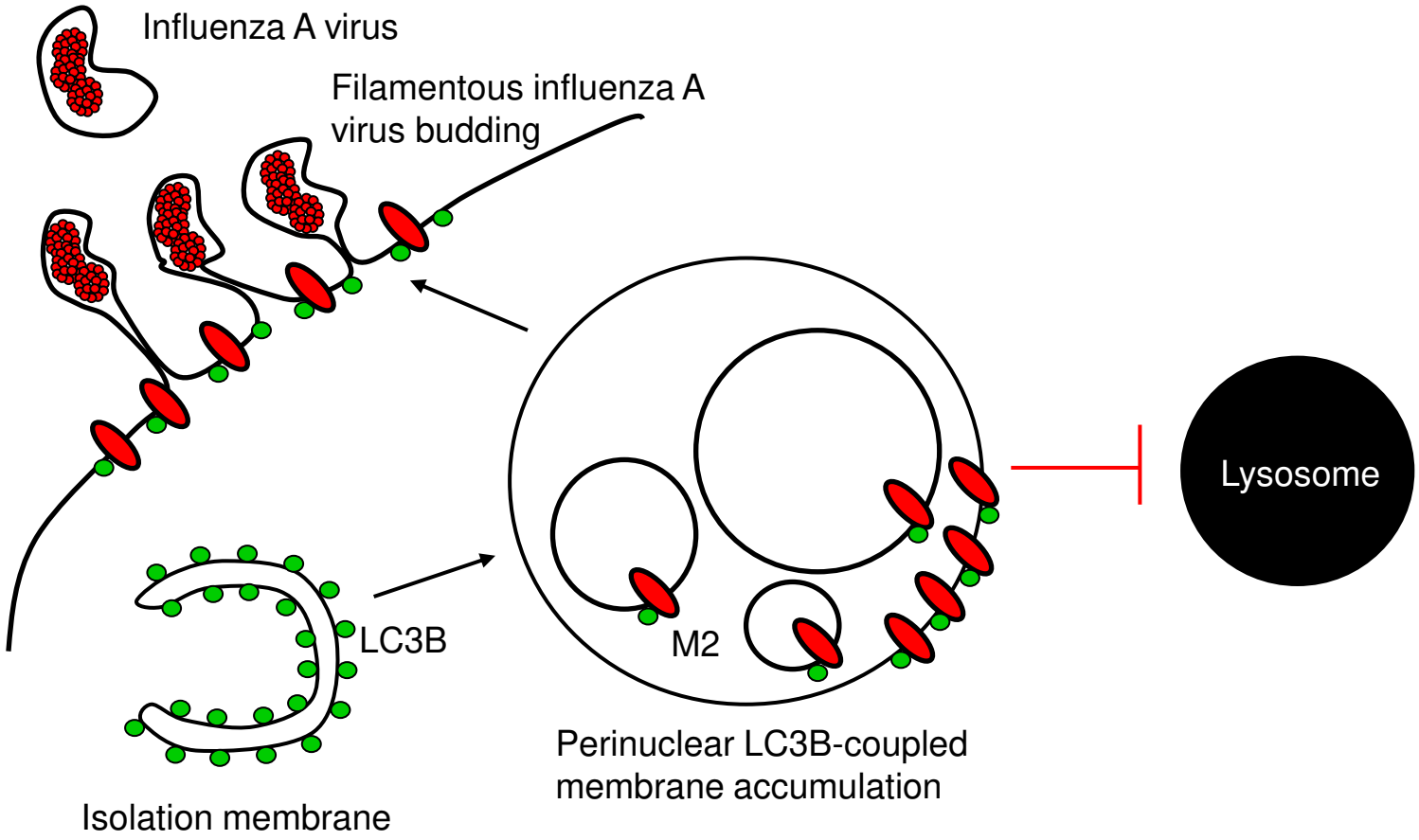
Influenza A virus
Filamentous influenza A virus budding
LC3B
M2
Lysosome
Isolation membrane
Perinuclear LC3B-coupled membrane accumulation

Figure 1